(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 January 2003 (09.01.2003)

PCT

(10) International Publication Number
WO 03/002064 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/20479

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/301,685 28 June 2001 (28.06.2001) US

(71) Applicant *(for all designated States except US)*: **ESSENTIAL THERAPEUTICS, INC.** [US/US]; 1365 Main Street, Waltham, MA 02451-1624 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MOE, Scott, T.** [US/US]; Three Carver Hill Road, Marlborough, MA 01752 (US). **CLEMENT, Jacob, J.** [US/US]; 74 Golden Run Rd., Bolton, MA 01740 (US). **FAERMAN, Carlos** [US/US]; 41 Mohawk Drive, Acton, MA 01720 (US). **PEROLA, Emanuela** [IT/US]; 127 Second Street, Apt. 2, Cambridge, MA 02141 (US). **NAVIA, Manuel, A.** [US/US]; 21 Washington Street, Lexington, MA 02421 (US). **ALA, Paul, J.** [CA/US]; 122 Bowdoin Street, Apt. 23, Boston, MA 02108 (US). **MAGEE, Andrew, S.** [US/US]; 21 Maple Street, Maynard, MA 01754 (US). **WILL, Paul, M.** [US/US]; 29 Pearl Street, Lunenburg, MA 01462 (US). **MARCHESE, Salvatore, A.** [—/US]; 32 Vining Street, Malden, MA 02148 (US). **GAZZANIGA, John, V.** [US/US]; Six Dominion Road, Worcester, MA 01605 (US).

(74) Agent: **HSI, Jeffrey, D.**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/002064 A2

(54) Title: NOVEL HETEROCYCLIC COMPOUNDS AS SELECTIVE BACTERIAL DHFR INHIBITORS AND THEIR USES THEREOF

(57) Abstract: This invention relates to novel heterocyclic compounds and to their use in the medical treatment or prophylaxis of bacterial infections, or their use as antiseptics, sterilizants, or disinfectants.

NOVEL HETEROCYCLIC COMPOUNDS AS SELECTIVE BACTERIAL DHFR INHIBITORS AND THEIR USES THEREOF

TECHNICAL FIELD OF THE INVENTION

This invention relates to novel heterocyclic compounds and to their use, for example, in the prophylaxis and or medical treatment of bacterial infections, and their use as antiseptics, sterilizants, or disinfectants.

BACKGROUND OF THE INVENTION

Research efforts in the fight against infection are ever increasing in order to keep pace with the proliferation of resistant microorganisms. Bacteria are continually mutating and evolving in a manner that convey increased resistance to existing therapeutics. This is particularly alarming in hospital settings, where a large proportion of observed pathogens are resistant to one or more standard treatment regimens. Additionally, the increase in the incidence of opportunistic infection in immunocompromised patients has contributed to the heightened urgency for novel therapies against infectious disease and disease symptoms. As a result, there is a very strong need for therapeutic agents that are more potent generally, more effective against resistant strains, and that cause fewer side effects than existing drugs. To address this increasing need, the focus of antimicrobial research includes the study of new agents that work by novel mechanisms, new agents that overcome known resistance profiles, and new or improved agents that act via known mechanisms.

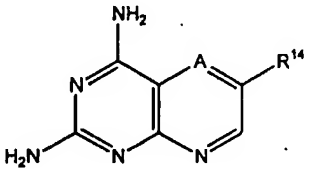
SUMMARY OF THE INVENTION

The invention relates to heterocyclic compounds, compositions comprising the compounds, and methods of using the compounds and compound compositions. The compounds and compositions comprising them are useful for treating disease or disease symptoms. The invention also provides for methods of making the compounds and methods for identifying compounds with desired biological activity.

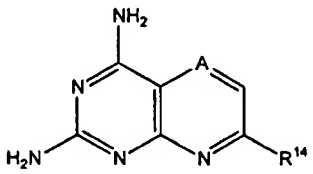
The invention is based on the discovery that certain heterocyclic compounds have potent antibacterial activity. Thus, this invention relates to novel heterocyclic compounds and to their use in the prophylaxis and/or medical treatment of bacterial infections or as

antiseptics, sterilizants, or disinfectants. The invention is further based on the discovery that certain heterocyclic compounds, including in general, substituted pteridinyl, quinazolinyl and pyrimidopyrimidinyl heterocycles, have potent antibacterial activity and are useful in the treatment of a variety of human diseases and microbial infections. As the heterocyclic compounds show selective inhibition of bacterial dihydrofolate reductase (bDHFR) versus human DHFR (hDHFR), the compounds of the invention can be useful in the treatment of microbial infections without human toxicity associated with inhibition of hDHFR.

One aspect of this invention relates to a compound of the following formulae:



I or

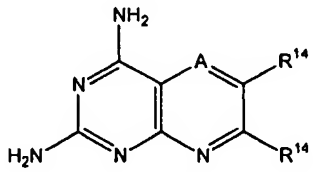


II

wherein A is N, CH, or CR^{15}; R^{14} is $-(CH_2)_n-X-Y$, wherein n is 1, 2, 3, 4, 5, or 6; X is O, NH, or NR^{15}; and Y is aryl or heteroaryl, wherein each Y is optionally substituted with 1-4 independent R^{16}. Each R^{15} is independently lower alkyl; each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $S(O)_2NR^{17}R^{17}$, $S(O)_2R^{17}$, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; each R^{17} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{18} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{19} is independently hydrogen, alkyl, aminoalkyl, aryl,

heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{20} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; and each R^{21} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, or heteroarylalkoxy.

In another embodiment, this invention relates to a compound of the following formula:



III

wherein A is N, CH, or CR^{15}; each R^{14} is independently $-(CH_2)_n-X-Y$, wherein n is 1, 2, 3, 4, 5, or 6; X is O, NH, or NR^{15}; and Y is aryl or heteroaryl, wherein each Y is optionally substituted with 1-4 independent R^{16}. Each R^{15} is independently lower alkyl; each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $S(O)_2NR^{17}R^{17}$, $S(O)_2R^{17}$, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; each R^{17} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl

or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{18} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{19} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; each R^{20} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; and each R^{21} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, or heteroarylalkoxy. In one aspect, the compounds (and compositions and methods relating to them) are those wherein each R^{14} is the same. In another aspect the compounds (and compositions and methods relating to them) are those wherein each R^{14} is different.

The term "halo" refers to any radical of fluorine, chlorine, bromine or iodine. The term "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C_1-C_{10} indicates that the

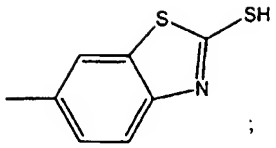
group may have from 1 to 10 (inclusive) carbon atoms in it. The term "lower alkyl" refers to a C_1-C_8 alkyl chain. The term "alkoxy" refers to an -O-alkyl radical. The term "alkylene" refers to a divalent alkyl (i.e., -R-). The term "alkylenedioxo" refers to a divalent species of the structure -O-R-O-, in which R represents an alkylene. The term "aminoalkyl" refers to an alkyl substituted with an amino. The term "mercapto" refers to an -SH radical. The term "thioalkoxy" refers to an -S-alkyl radical.

The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "arylalkyl" or the term "aralkyl" refers to alkyl substituted with an aryl. The term "arylalkoxy" refers to an alkoxy substituted with aryl.

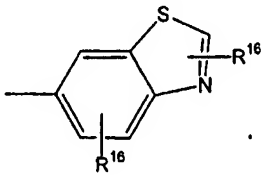
The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like. The term "heteroarylalkyl" or the term "heteroaralkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

A carbon-, nitrogen-, sulfur- halogen- and/or oxygen-containing function group is a substituted or unsubstituted, linear, branched, or cyclic, alkyl, alkenyl, alkynyl, aryl, aralkyl, or alkaryl group, or a derivative of one or more of these groups where heteroatoms are substituted for one or more of the carbon and/or hydrogen atoms (e.g., amino groups, alkylamino groups, hydroxyl and alkoxyl groups, thiol groups, halogens, nitro groups, phenolic groups, or other substituted aromatic or aliphatic groups)).

Other embodiments include compounds of any of the formulae described herein wherein A is N; those wherein n is 1; those wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}; those wherein Y is phenyl; those wherein Y is phenyl substituted with 1-4 independent R^{16}; those wherein Y is

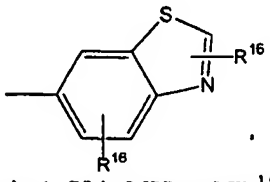


optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is halo, alkyl, alkylenedioxo, aryl, aralkyl, nitro, hydroxy, mercapto, amine, alkoxy, arylalkoxy, or heteroarylalkoxy; those wherein Y is



those wherein A is CH or CR^{15}; those wherein each R^{16} is independently $C(O)NR^{19}R^{20}$ [each R^{19} is independently aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl; and each R^{20} is independently aminoalkyl, aryl, or arylalkyl; each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$]; those wherein Y is heteroaryl; those wherein n is 1 and X is NH or NR^{15}, and Y is phenyl substituted with one R^{16}, in which R^{16} is not –COOH; and those wherein n is 1 and X is NH or NR^{15}, and Y is phenyl substituted with 2-4 independent R^{16}.

Other embodiments include compounds of any of the formulae described herein wherein n is 1, and X is O; those wherein n is 1, X is NH or NR^{15}, and R^{15} is CH_3; those wherein n is 1, X is NH or NR^{15} (R^{15} is CH_3), and Y is phenyl, optionally substituted with 1-

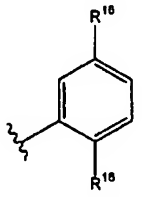


4 independent R^{16}; those wherein n is 1, X is NH or NR^{15}, and R^{15} is CH_3, and Y is optionally substituted with 1-4 independent R^{16}, each R^{16} being halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy,

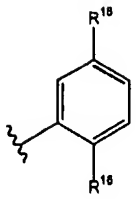
mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; and those wherein Y is phenyl (optionally substituted with 1-4 independent R^{16}) and A is N.

Other embodiments include compounds of any of the formulae described herein wherein n is 1, X is O, and A is N; those wherein n is 1, X is O, Y is phenyl, optionally substituted with 1-4 independent R^{16}; those wherein n is 1, X is O, Y is phenyl substituted with 1-4 independent R^{16}; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is independently $C(O)OR^{18}$; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is independently $C(O)OR^{18}$ and each R^{18} is H or alkyl; those wherein n is 1, X is NH or NR^{15} (wherein R^{15} is CH_3), A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is independently $C(O)NR^{19}R^{20}$.

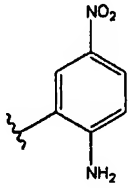
Other embodiments include compounds of any of the formula described herein wherein n is 1, X is O, A is N, Y is phenyl, optionally substituted with 1-4 independent R^{16}; those wherein n is 1, X is O, A is N, Y is



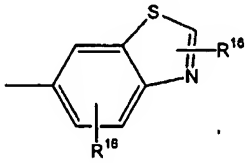
those wherein n is 1, X is O, A is N, Y is



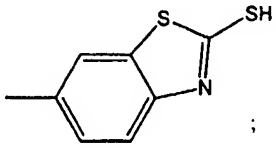
wherein each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; those wherein n is 1, X is O, A is N, and Y is



those wherein n is 1, X is NH or NR^{15} (R^{15} is CH_3), A is N, Y is

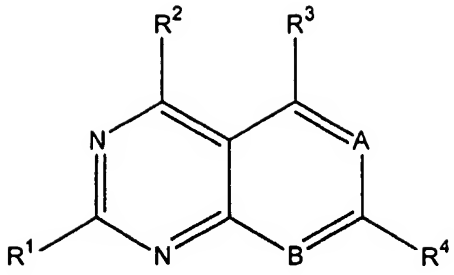


optionally substituted with 1-4 independent R^{16}, each R^{16} being halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$; those wherein n is 1, X is NH or NR^{15} (R^{15} is CH_3), A is N, Y is



or any of the compounds in Table 1.

In another aspect, the invention relates to a compound having the formula:



III

wherein

A and B are independently selected from the group consisting of N and CR^7, wherein R^7 is hydrogen or a carbon-, nitrogen-, sulfur- halogen- and/or oxygen-containing function group;

R^1 and R^2 are $-NR^5R^6$ groups, wherein R^5 and R^6 are independently hydrogen or carbon-containing functional groups;

R^3 is hydrogen;

R^4 is a carbon-, nitrogen-, sulfur-, halogen-, and/or oxygen-containing functional group,

provided that,

if A and B are both nitrogen and R^5 and R^6 are both hydrogen, then R^4 is not $-NH_2$, -N(H)(methyl), -N(H)(butyl), -N(H)(hexyl), -N(H)(phenyl), -N(H)(benzyl), $-N(H)(NH_2)$, $-N(H)(CH_2CH_2OH)$, $-N(CH_2CH_2OH)_2$, phenyl, N-piperidinyl, or -S(ethyl);

if A is CH, B is nitrogen, and R^5 and R^6 are both hydrogen, then R^4 is not methyl, isobutyl, phenyl, 4-methylphenyl, 4-chlorophenyl, 4-bromophenyl, 2-(2,5-dimethoxyphenyl)-ethyl, or $-CH(OCH_3)_2$; and

if A and B are both CH groups, then R^4 is an amino group other than $-NH_2$, (3,4-dichlorophenyl)methylamino, or (3,4-dichlorophenyl)methyleneimino.

In other aspeacts, the compounds are those of formula III wherein,

R^5 and R^6 are independently hydrogen; and

A and B are each independently N;

those of any of the formulae herein wherein,

R^4 is NR^7R^8;

those of any of the formulae herein wherein,

R^4 is NR^7R^8;

R^7 is C1-C6 alkyl substituted with aryl or heteroaryl; and

R^8 is C1-C6 alkyl optionally substituted with alkenyl, hydroxyl, alkoxy, cycloalkyl, or aryl;

those of any of the formulae herein wherein,

R^7 is C1-C6 alkyl substituted with aryl;

R^8 is C1-C6 alkyl;

those of any of the formulae herein wherein,

R^7 is C1-C6 alkyl substituted with heteroaryl;

R^8 is C1-C6 alkyl;

those of any of the formulae herein wherein,

R^7 is C1-C6 alkyl substituted with aryl;

R^8 is C1-C6 alkyl substituted with hydroxyl or alkoxy;

those of any of the formulae herein wherein

R^4 is NR^7R^8;

those of any of the formulae herein wherein

R^4 is NR^7R^8;

R^7 is C1-C6 alkyl;

R^8 is C1-C6 alkyl substituted with cycloalkyl;

those of any of the formulae herein wherein,

R^4 is NR^7R^8;

R^7 is independently C1-C6 alkyl substituted with aryl;

R^8 is independently C1-C6 alkyl substituted with aryl;

those of any of the formulae herein, wherein

R^7 is hydrogen, alkyl, cycloalkyl, aryl, halogen, thioalkyl, hydroxy, alkoxy, amino, alkyl, or NR^5R^6; wherein each R^5 and R^6 on the nitrogen atom independently is hydrogen, alkyl, cycloalkyl, aryl, arylalkyl, heteroarylalkyl, or alkylcarbonyl;

those of any of the formulae herein, wherein

R^7 is C1-C6 alkyl substituted with naphthyl, which is optionally substituted with alkyl, halo, hydroxy, alkoxy, thioalkyl, or amino;

those of any of the formulae herein, wherein the naphthyl group is substituted at the 2- or 4-position;

those of any of the formulae herein, wherein

R^8 is methyl or ethyl;

those of any of the formulae herein, wherein

R^7 is C1-C6 alkyl substituted with benzothienyl, which is optionally substituted with alkyl, halo, hydroxy, alkoxy, thioalkyl, or amino;

those of any of the formulae herein, wherein R^8 is methyl or ethyl.

Other aspects of this invention relate to a composition having a compound of any of the formulae described herein and a pharmaceutically acceptable carrier; or a compound of any of the formulae described herein, an additional therapeutic agent, and a pharmaceutically acceptable carrier; or a compound of any of the formulae described herein, an additional therapeutic agent, and a pharmaceutically acceptable carrier, wherein the additional therapeutic agent is an antibacterial agent.

Yet another aspect of this invention relates to a method of treating a subject (e.g., mammal) infected with one or more bacteria (including, but not limited to urinary tract infections, systemic and topical infections, sepsis, antibiotic mediated colitis, ulcers of the gastrointestinal tract, topical disinfectant, antiseptic, sterilizant, wound care, and surgical cleansing). The method includes administering to the subject (including a subject identified as in need of such treatment) an effective amount of a compound described herein, or a composition described herein to produce such effect.

The compounds and compositions herein are also useful as antiparasitic agents. Another aspect of this invention relates to a method of treating a subject (e.g., a mammal) infected with one or more parasites. The method includes administering to the subject (including a subject identified as in need of such treatment) an effective amount of a compound described herein, or a composition described herein to treat parasites, or disease, infection, or symptoms thereof.

The invention also relates to a method of making a compound described herein. The method includes taking a 2,4-diamino pteridinyl (or a 2,4-diaminopyrimidopyrimidinyl) compound and reacting it with one or more chemical reagents in one or more steps to

produce a compound described herein. Alternatively, the method includes taking any one of the intermediate compounds described herein and reacting it with one or chemical reagents in one or more steps to produce a compound described herein.

The invention further relates to a product (i.e., a compound of any of the formulae) made by the method described above.

Also within the scope of this invention are a method for identifying a compound having antibacterial activity which includes: a) assessing the structure of a compound of a formula herein; b) procuring a derivative compound of the compound from step a); and c) assessing the antibacterial activity of the derivative compound; and a method for identifying a compound having antibacterial activity which includes: a) taking a candidate compound; b) assessing the binding affinity of the candidate compound in a model of the DHFR (e.g., bDHFR, hDHFR) enzyme; and c) assessing the antibacterial activity of the candidate compound. Assessing antibacterial activity can be performed by a variety of procedures known in the art, including those delineated herein.

A compound that can inhibit bacterial DHFR selectively over human DHFR can provide an advantageous treatment option for bacterial disease or disease symptoms. 6-substituted pteridines have been described in the literature as potent inhibitors of either human DHFR (e.g. methotrexate, used as anti-cancer agent) or bacterial DHFR (e.g. Trimetrexate used for treatment of bacterial and parastic infections), Existing treatments with compounds such as trimetrexate have severe side effects and toxicities associated with inhibition of DHFR. The compounds in this invention are potent bDHFR inhibitors and significantly less active against hDHFR. These compounds can therefore selectively affect (e.g., kill, inhibit growth/proliferation of) microorganisms through the inhibition of bDHFR with reduction or elimination of substantial side effects to the subject (e.g., human, animal, mammal), including those associated (in whole or in part) with inhibition of hDHFR.

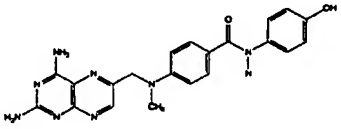
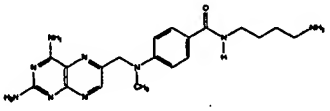
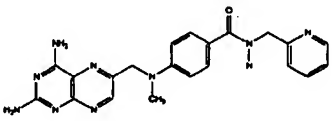
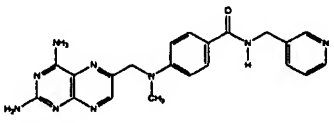
Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject or antiseptic, wound dressing impregnation, sterilizant, or disinfectant applications).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

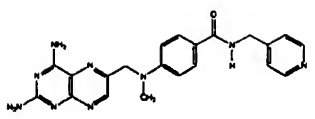
Specific features and advantages of the invention will be apparent from the following detailed description, and from the claims.

The invention also relates to the specific compounds exemplified herein. Thus one embodiment of the invention is any compound specifically delineated herein, including the exemplary compounds listed below:

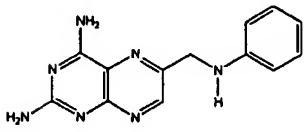
Table 1. Compounds and their IDs

ID	STRUCTURE
1	
2	
3	
4	

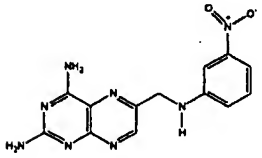
5



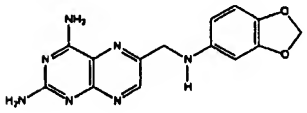
6



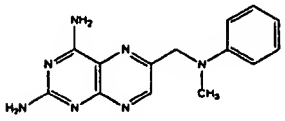
7



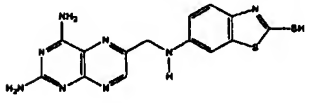
8



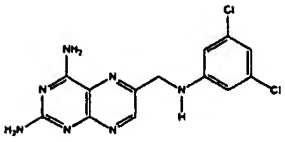
9



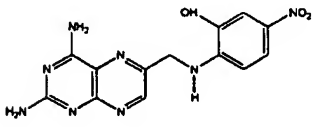
10

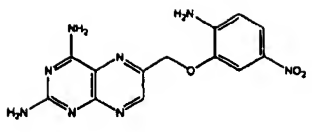


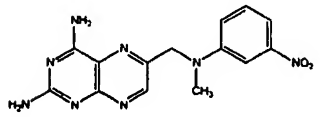
11

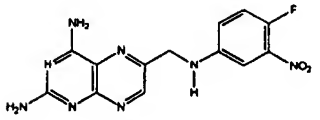


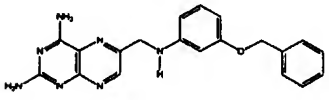
12

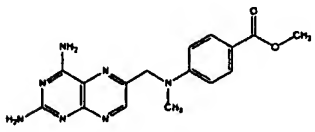


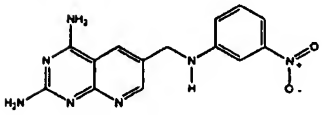
13 

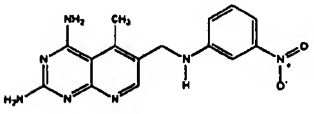
14 

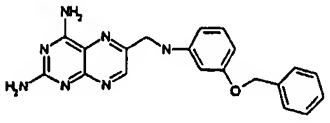
15 

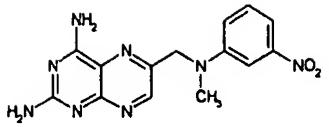
16 

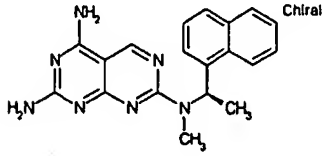
17 

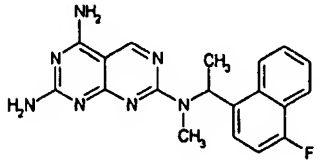
18 

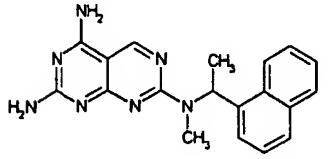
19 

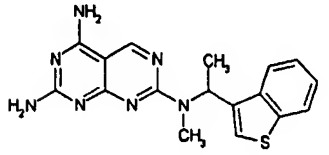
20 

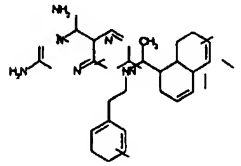
21 

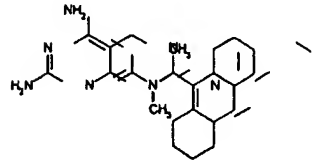
22 

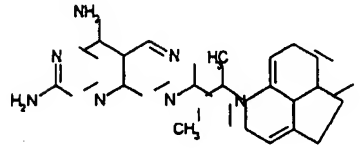
23 

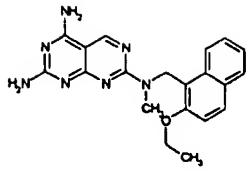
24 

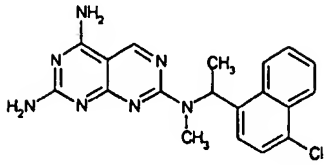
25 

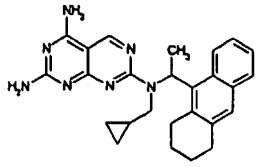
2 6 

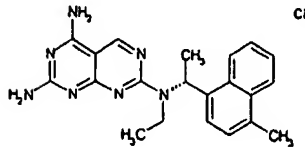
2 7 

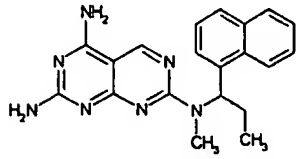
2 8 

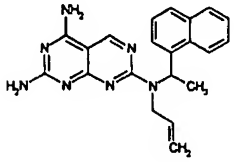
29 

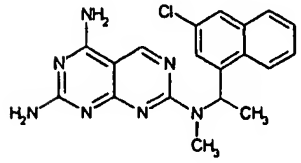
30 

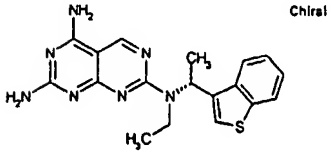
31 

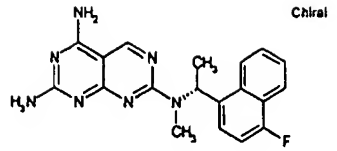
32 

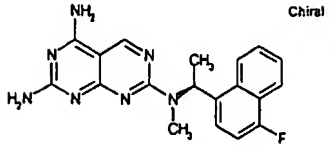
33 

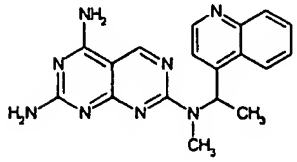
34 

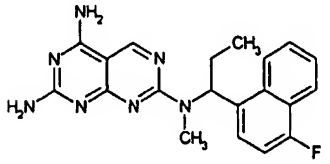
35 

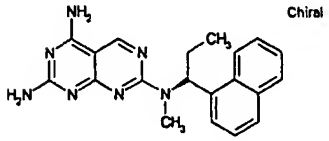
36 

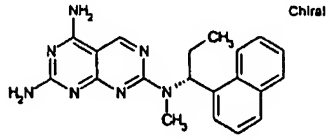
37 

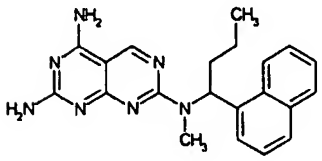
38 

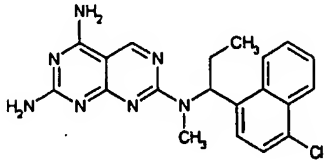
39 

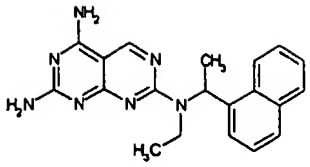
40 

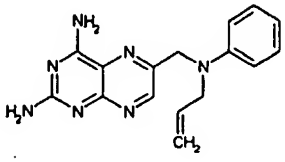
41 

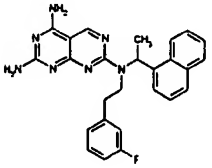
42 

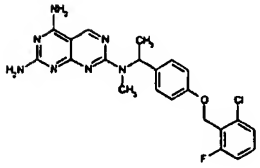
43 

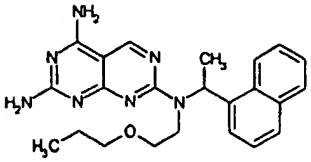
44 

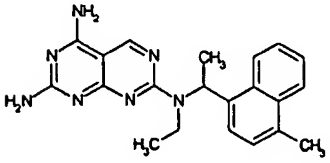
45 

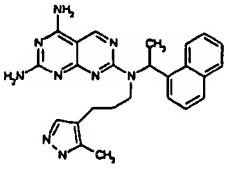
46 

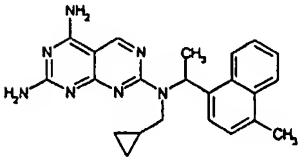
47 

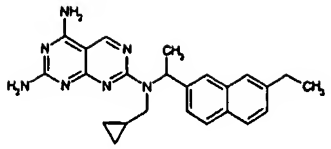
48 

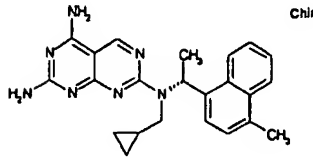
49 

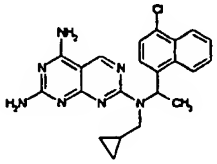
50 

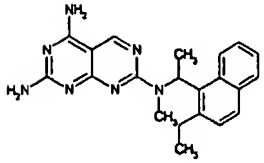
51 

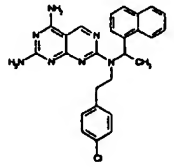
52 

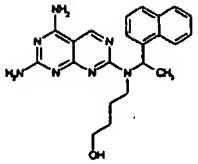
53 

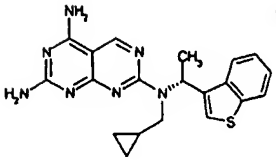
54 
Chiral

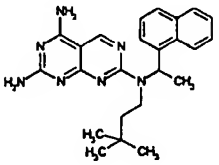
55 

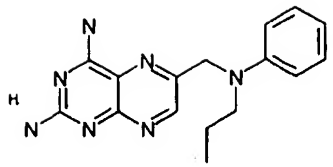
56 

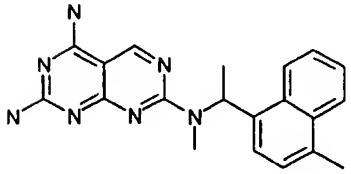
57 

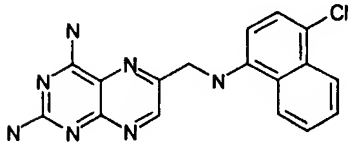
58 

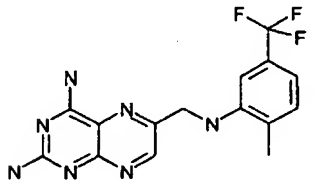
59 
Chiral

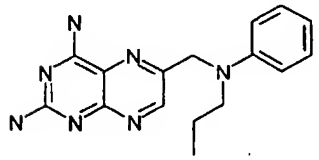
60 

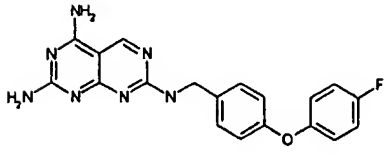
61 

62 

63 

64 

65 

66 

The following table of compounds of one aspect of the invention have potent antimicrobial activity against S. aureus bacteria and are anticipated to have physical-chemical properties similar to those of orally absorbed and bioavailable drugs and drug-like compounds. Antibacterial activity for a variety of bacterial species are shown (S. aureus, S.

pneumoniae, E. faecalis, M. catarrhalis, and H. influenzae). The yeast cell (S. cerevisiae) in this panel of organisms is an indicator of potential cytotoxicity in mammalian cells. All analogs assayed showed "toxicity" at higher concentrations than 32 ug/mL.

The following table of compounds of one aspect of the invention have potent antimicrobial activity against S. aureus bacteria and are anticipated to have physical-chemical properties similar to those of orally absorbed and bioavailable drugs and drug-like compounds. MIC's are given in mg/ml

Table of representative compounds of the invention

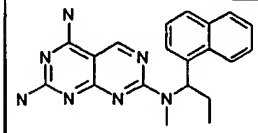
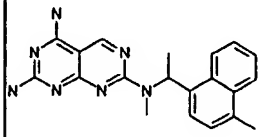
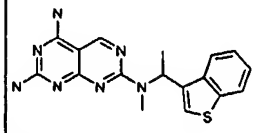
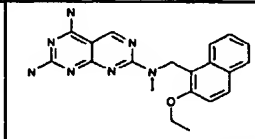
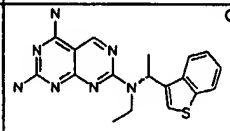
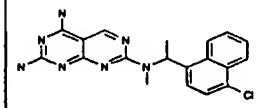
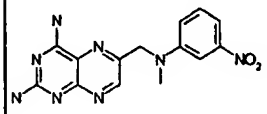
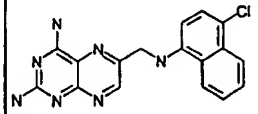
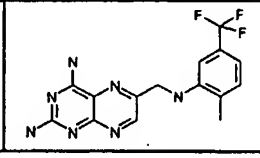
Corporate ID	bDHFR Ki/ (μM) avg	S. aureus (μg/ml) avg
20	0.062	0.5
21	0.082	<0.125
22	0.002	<0.125
23	0.027	1
24	0.015	0.25

25	0.004	0.25
26	0.02	1
27	0.008	0.5
28	0.02	<0.125
29	0.02	<0.125
30	0.028	<0.125
31	0.024	1
32	0.011	0.5
33	0.012	0.015

34	0.014	1
35	0.037	0.5
36	0.005	<0.125
37	0.003	<0.125
38	0.06	0.5
39	0.015	0.25
40	0.004	<0.125
41	0.037	<0.125
42	0.004	<0.125
43	0.004	<0.125

44	0.004	<0.125
45	0.008	2
46	0.093	2
47	0.023	2
48	0.007	2
49	0.02	2
50	0.004	2
51	0.022	2
52	0.022	2

53	0.03	2
54	0.01	2
55	0.019	2
56	0.093	2
57	0.011	2
58	0.015	2
59	0.005	2
60	0.029	2
61	0.013	2

ID#	bDHFR (nM)	Structure	*S. aureus* MIC	*S. pneumoniae* MIC	*E. faecalis* MIC	*M. catarrhalis* MIC	*H. influenzae* MIC	*S. cerevisiae* MIC
33	12		<0.125	,.125	1, 32	1	8	>64
62	802		<0.125	2	>4	4	64	32
25	<4		0.5	1	32	4	16	>64
29	<20		0.125	0.5	16	1, 2	64	64
36	5	 Chiral	1	1, 8	32	0.5, 1	16	>64
30	24		0.13	2	8	2	64	64
21	82		0.25	0.125	0.015	1	1	>64
63	104		0.5	0.25	0.25	0.25	32	64
64	385		0.125	0.125	0.125	0.125	64	>64

The compounds of this invention may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting materials. In general, the compounds of the formulae described herein are conveniently obtained via methods illustrated in the schemes and the Examples herein.

Thus, one embodiment relates to a method of making a compound of the formulae described herein, comprising synthesizing any one or more intermediates illustrated in the synthetic schemes herein and then converting that intermediate(s) to a compound of the formulae described herein. Another embodiment relates to a method of making a compound of the formulae described herein, comprising synthesizing any one or more intermediates illustrated in the examples herein and then converting that intermediate(s) to a compound of the formulae described herein. Another embodiment relates to a method of making a compound of the formulae described herein, comprising synthesizing any one or more intermediates illustrated in the synthetic schemes herein and then converting that intermediate(s) to a compound of the formulae described herein utilizing one or more of the chemical reactions described in the synthetic schemes or examples herein. Nucleophilic agents are known in the art and are described in the chemical texts and treatises referred to herein. The chemicals used in the aforementioned methods may include, for example, solvents, reagents, catalysts, protecting group and deprotecting group reagents and the like. The methods described above may also additionally comprise steps, either before or after the steps described specifically herein, to add or remove suitable protecting groups in order to ultimately allow synthesis of the compound of the formulae described herein.

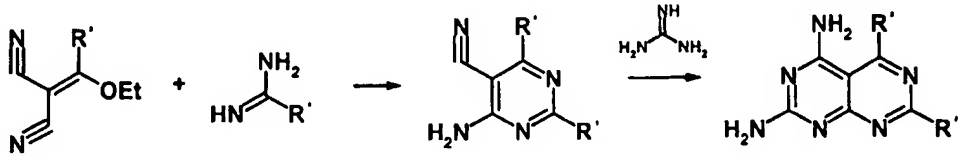
As can be appreciated by the skilled artisan, the synthetic schemes herein are not intended to comprise a comprehensive list of all means by which the compounds described and claimed in this application may be synthesized. Further methods will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps described above may be performed in an alternate sequence or order to give the desired compounds. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's*

Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995) and subsequent editions thereof.

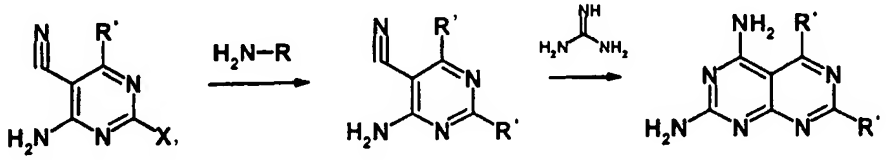
Discussion of General Synthetic Methodologies used in the preparation of analogs.

In general, compounds of the invention described herein can be prepared by utilizing standard methods known to those skilled in the art of organic synthesis and using standard methods and techniques described in the literature.

Synthesis of Pyrimidopyrimidines



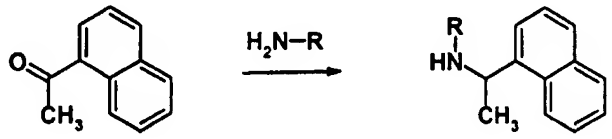
Pyrimidopyrimdine compounds of the invention can be prepared using a variety of synthetic strategies. The pyrimidopyrimidine ring system can be synthesized in a multi-step reaction sequence starting from an appropriately substituted amidine (R7-C=NHNH2) and an R5-substituted alkoxylmethylenemalonitrile. The resulting cyanoaminopyrimidine can be condensed with guanidine to form the pyrimidopyrimine ring system. In the case where R7 in the cyanoaminopyrimidine is either –Cl, -Br, -S-lower alkyl, these leaving groups can be substituted with substituted nitrogen or oxygen nucleophiles to provide R7-N(or O)-substituted alkyl or aryl intermediates. These intermediates can be cyclized to their corresponding pyrimidopyrimidines with appropriately substituted at the 7-position.



Another method to synthesize 7-aminosubstituted pyrimidopyrimidines is through the nucleophilic attack of amines on 6-amino-2-bromopyrimidine-5-carbonitrile (chloro or

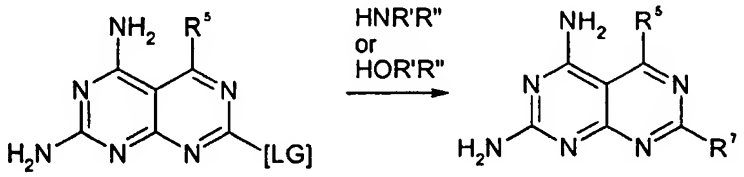
thiomethyl, or thioethyl can also be used as leaving groups at the 2-position), and subsequent cyclization of the resulting, appropriately substituted cyanoaminopyrimidine with guanidine.

If the attacking, appropriately substituted nucleophilic amine is not commercially available, then it can be prepared using standard methods in organic chemistry. One such standard method used in preparing compounds in this application is by reductive-amination.



In this well-known procedure, an aldehyde or ketone is condensed with an appropriately substituted amine in the presence of a mild reducing agent such as sodium cyanoborohydride or zinc cyanoborohydride.

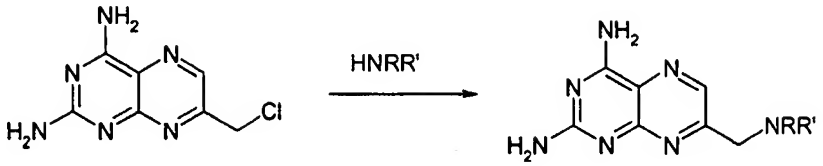
Direct heterocyclic substitution methods



Compounds of the invention can also be prepared by the aromatic nucleophilic displacement of leaving groups on the 7-position of 2,4-diaminopyrimidopyrimidine. These leaving groups [LG] include, but are not limited to: -Cl, -Br, $-SCH_3$, $-SC_2H_5$, and $-N(CH3)_3$. The nucleophile used in the displacement reaction can be –N-alkyl, -N-aryl, or substituted alkyl or aryl amines, or –O-alkyl, -O-aryl, or substituted alcohols or phenols.

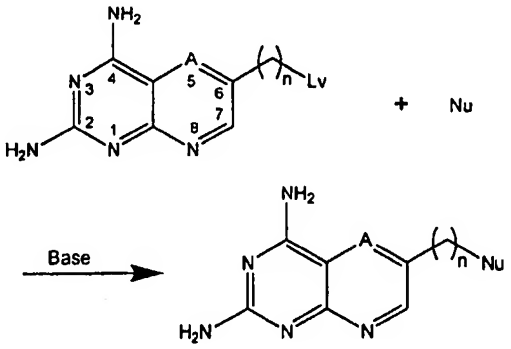
Synthesis of Pterin Analogs

In general either 6- or 7-substituted pterin analogs can be prepared by the reaction of an activated reagent such as 6- or 7-chloromethyl pterin with nucleophiles such as amines, and by various other methods described in the literature other functional groups at the 6- and 7-position of pterin such as bromomethyl, iodomethyl, hydroxymethyl, activated hydrodroxymethyl, carbonyl, activated carbonyl, hydroxy, chloro, bromo, or methyl can be used as synthetic reagents for the preparation of 6- or 7-substitued analogs.



The above general reaction pathway can be used to synthesize a broad range of 7-substituted pteridine analogs. In a general procedure, 7-chloromethyl and an appropriate amine are reacted in an appropriate solvent such as DMF or 2-methoxyethanol for as long as needed as determined by analysis of the reaction mixture by HPLC, TLC, or NMR. The solvent is then removed and the product purified by an appropriate method, usually in the form of precipitation, recrystallization, re-precipitation of the salt by base, or through chromatography.

2,4-Diamino pteridinyl substituted at C-6 with a leaving group-containing alkyl in a suitable solvent is coupled with an amine, aniline, phenol, or alkoxide, which functions as a nucleophile, in the presence of a suitable base. (See the reaction scheme below.) A leaving group is a chemical group that can be detached from a molecule during a reaction. The leaving group may be of an ionic form after detachment from the original molecule (e.g., -OH, -SMe, -OAc, -OTosyl, Cl-, Br-, I-) or can be of neutral form (e.g., H_2O, HOAc). The resulting product is then isolated and purified using standard and known techniques.



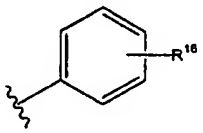
Lv = Leaving group
Nu = nucleophile

Similarly, a diaminopyrimidinyl pyridinyl compound can be treated using similar methodology to prepare a compound herein in which A is CH or C-lower alkyl.

The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers

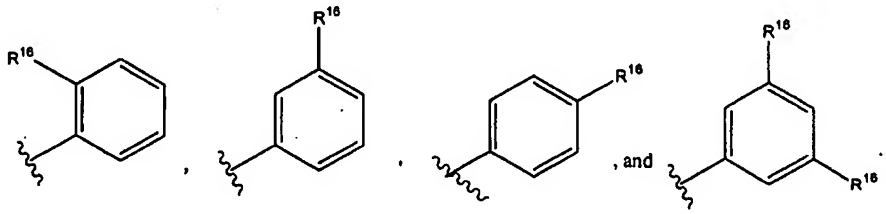
and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention. The compounds of this invention may also be represented in multiple tautomeric forms (see illustration), in such instances, the invention expressly includes all tautomeric forms of the compounds described herein (e.g., alkylation of a ring system may result in alkyation at multiple sites, the invention expressly includes all such reaction products). All such isomeric forms of such compounds are expressly included in the present invention. All crystal forms of the compounds described herein are expressly included in the present invention.

Substituents on ring moieties (e.g., phenyl, thienyl, etc.) may be attached to specific atoms, whereby they are intended to be fixed to that atom, or they may be drawn unattached to a specific atom (see below), whereby they are intended to be attached at any available atom that is not already substituted by an atom other than H (hydrogen). For example, a structure drawn as:



is intended to encompass all of (but is not limited to) the following structures:

All references cited herein, whether in print, electronic, computer readable storage media or other form, are expressly incorporated by reference in their entirety, including but not limited to, abstracts, articles, journals, publications, texts, treatises, internet web sites, databases, patents, and patent publications.



Formulations, compositions, and prodrugs

As used herein, the compounds of this invention, including the compounds of formulae described herein, are defined to include pharmaceutically acceptable derivatives or prodrugs thereof. A "pharmaceutically acceptable derivative or prodrug" means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a compound of

this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species. Preferred prodrugs include derivatives where a group which enhances aqueous solubility or active transport through the gut membrane is appended to the structure of formulae described herein.

The compounds of this invention may be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological compartment (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-$(alkyl)_4^+$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

In vitro Assays for Antibacterial Activity

The compounds can be screened for antibacterial activity using standard methods.

In one example, illustrated below, broth microdilution techniques are used to measure *in vitro* activity of the compounds against a given bacterial culture, to yield minimum inhibitory concentration (MIC) data. The MICs of some representative compounds are provided in Table 2 below.

Table 2. MIC (μg/mL) of some compounds for various bacteria (data given in mg/l n.t.: not tested).

Compound ID	E. coli	S. aureus	E. faecalis	S. pneumoniae	M. catarrhalis	H. influenzae	P. aeruginosa	S. cerevisiae
1	>64	16-64	16-64	<2	2-8	2-8	>64	>64
3	>64	2-8	2-8	2-8	2-8	2-8	>64	>64
4	>64	16-64	2-8	2-8	16-64	2-8	>64	>64
5	>64	16-64	2-8	2-8	2-8	2-8	>64	>64
6	>64	2-8	2-8	2-8	2-8	2-8	>64	>64
7	>64	<2	<2	<2	<2	<2	>64	>64
8	>64	2-8	2-8	2-8	2-8	2-8	>64	>64
9	>64	2-8	<2	<2	2-8	<2	>64	>64
10	>64	2-8	<2	2-8	<2	16-64	>64	16-64
11	>64	<2	<2	<2	<2	>64	>64	>64
12	>64	>64	n.t.	<2	16-64	2-8	>64	>64
14	>64	<2	<2	<2	<2	<2	>64	>64
15	>64	<2	<2	<2	<2	<2	>64	2-8

16	>64	<2	<2	<2	<2	>64	>64	>64
17	>64	<2	<2	<2	<2	<2	>64	>64

Microdilution Antimicrobial Susceptibility Test Assay

Stock solutions of tested compounds were prepared in *N,N*-dimethylformamide (DMF) at a concentration of 5 mg/mL. Working solutions of the tested compounds were then prepared from the stock solutions, in Mueller-Hinton broth (MHB) with a starting concentration of 64 µg/mL.

Bacterial inocula were prepared from overnight culture (i.e., one fresh colony from agar plate in 5 ml MHB; *H. influenzae* was grown in MHB with the addition of yeast extract, haematin, and NAD), centrifuged 2 x 5 min/3000 rpm (for *S. pneumoniae* and *H. influenzae*, 2 x 10 min/3000 rpm), and dispensed in 5 ml of fresh MHB each time, such that the bacterial suspension is diluted to obtain 100 colony forming units (cfu) in a microplate well (100 µl total volume).

Microplate wells were filled with two-fold dilutions of test compound (50 µl), starting with 64 µg/ml. Wells were then filled with 50 µl of bacterial inoculum (final volume: 100 µl/well). The plates were incubated at 37 °C for 18-24 hours (*S. pneumoniae* was grown in a CO_2-enriched atmosphere).

The optical density of each well at 590 nm (OD_{590}) was then measured with a TECAN SpectroFluor Plus®, and minimum inhibitory concentration (MIC) was defined as the concentration that showed 90% inhibition of growth.

Biochemical Assays to Determine Activity Against Bacterial and Human DHFR

Materials and Methods:

Human and Bacterial DHFR: E. *coli* DHFR was kindly provided by Eric Brown of MacMaster University.

Cloning, expression, purification of human DHFR

The human DHFR cDNA was cloned by PCR from the Human Universal Quick-clone™ cDNA purchased from Clontech. PCR primers were designed from the human sequence deposited in GenBank (1) under Accession number XM_003991. The primer

sequences are as follows and result in the amplification of a 186 amino acid hDHFR coding region:

oAlt-92 (5') PCR primer: gatcgatcgatccatatggttggttcgctaaactgc

oAlt-93 (3') PCR primer: gatcgatcaagcttcattaatcattcttctcatatacttc

The PCR fragment was digested with restriction enzymes NdeI and HindIII, and then ligated into a derivative of the pKK223-3 expression vector (Amersham Pharmacia). The final expression vector (pFW96.2) was transformed into *E. coli* Top10 F' and hDHFR protein was induced by the addition of 1mM IPTG followed by incubation at 37°C for 4 hrs. Recombinant human DHFR was purified based on a previously published procedure (2).

Enzyme Assays

Dihydrofolic acid and NADPH were purchased from Sigma. Enzyme assays were adapted for microtitre format from a previously published procedure (2, 3). All assays were performed in a 250µl volume in 96-well microtitre plates. Typical assays consisted of 20nM DHFR (human or *E. coli*), 100µM NADPH, and 50µM dihydrofolic acid in 100mM HEPES pH 8, 10mM KCl, and 10mM $MgCl_2$. The assay was monitored spectrophotometrically for the conversion of NADPH to NADP+, which results in a decrease in absorbance at 340 nm. The extinction coefficient for the reaction at 340 nm (12,300 $M^{-1}cm^{-1}$) (4) was used to convert absorbance to concentration of product.

K_i Determinations

Compounds were serially diluted in DMSO to concentration ranges from 25mM to 1µM. Five microliters of inhibitor compound in DMSO was added to 120µl of enzyme solution (40nM DHFR and 200µM NADPH). The reaction was initiated by the addition of 125µl of 100µM dihydrofolic acid. The final concentrations in the assay were 20nM DHFR, 100µM NADPH, 50µM dihydrofolic acid, and a range of 500µM to 20nM inhibitor compound. The final DMSO concentration was 2% of the total volume. Reactions were monitored as described above.

Observed rates (nM/sec) were plotted versus inhibitor concentration on a log scale and K_i values were determined by fitting the resulting curves to the following equation:

$V = (V_{max}[S])/(K_m(1 + I/K_i) + [S])$, where S = dihydrofolate, K_m = Michaelis constant for dihydrofolate, I = inhibitor concentration.

The compounds of the invention are generally more potent for the inhibition of the bacterial DHFR (bDHFR) enzyme over human DHFR (hDHFR) enzyme. This selectivity of inhibition is a useful property in antibacterial drugs. The antibacterial drug Trimethoprim is an example. Trimethprim inhibits bacterial DHFR at <0.002 uM; Trimethoprim is essentially ineffective at inhibiting human DHFR enzymes. Inhibition of human DHFR causes profound side-effects in human patients. Compounds with a high human versus bacterial DHFR inhibition ratio can be considered "safer" with regards to their primary mechanism of side-effect; i.e., the inhibition of hDHFR. Methotrexate is a drug used in the treatment of cancer. Its primary mechanism of action is through the inhibition of hDHFR enzymes present in cancerous cells. Many side-effects of this form of cancer-chemotherapy are the result of inhibition of hDHFR. Drugs like trimetrexate (not shown) are antimicrobial agents (6-substituted-quinazoline) that have significant hDHFR inhibition. It is necessary to administer/co-administer what is termed a "rescue" drug, e.g., leukovorin, to the patient to combat the powerful, deadly side-effects of hDHFR inhibition. Thus in one embodiment, a compound of a formulae herein is one that inhibits bacterial DHFR at a much lower dose (high potency) than that which shows inhibition of hDHFR enzymes, or has a therapeutic index (i.e., the ratio of hDHFR Ki / bDHFR Ki) greater than 10 (e.g., > 50, >100, >1000, >10,000, >20,000, >25,000).

A comparison of bacterial versus human DHFR Ki values of representative compounds herein are summarized below in Table 3 below. The high selectivity of the compounds of the invention for bacterial over human DHFR inhibitions indicates that these analogs may have a high therapeutic index with respect to the ratio of efficacy (antimicrobial activity) in comparison with toxicity (human side-effects associated with hDHFR inhibition).

Table 3. Comparison of bacterial versus human DHFR Ki values (nM) of representative compounds
n.t.: not tested)

Compound ID	bDHFR Ki	hDHFR
1	<100	500-999

2	100-499	1000-4999
3	<100	1000-4999
4	<100	500-999
5	<100	500-999
6	1000-4999	>5000
7	100-499	>5000
8	1000-4999	>5000
9	100-499	>5000
10	100-499	>5000
11	500-999	>5000
12	100-499	1000-4999
13	500-999	>5000
14	<100	>5000
15	100-499	>5000
16	<100	>5000
17	<100	n.t.
21	<100	>5000
22	<2	> 75,000
66	<1000	> 500,000

***In vivo* Assays for Antibacterial Activity**

The compounds can also be tested for antibacterial efficacy in laboratory animals. These *in vivo* studies include, but are not limited to, systemic and topical models of infection, urinary tract infection models, helicobacter infections including ulcers of the gastrointestinal tract, sepsis, antibiotic mediated colitis and wound care. The compounds of the invention can also be evaluated in animals to assess their pharmacokinetic profiles, such as oral bioavailability, oral absorption, chemical half-life, identification of metabolites, serum levels at various times, and rate of excretion, for example.

Systemic bacterial infection animal models

Systemic models of infection are described in the literature. The following conditions can be used to assay the compounds in this application. Bacteria are grown in Mueller-Hinton agar at 37 °C during 24 h. For each experiment, a bacterial suspension is prepared by inoculating 4 – 5 bacterial colonies onto Mueller-Hinton broth (MHB) and by incubating at 37°C for 24 hours to yield approximately 10^9 CFU/ml. BalbC female mice are supplied by Charles River (Deutschland). Animals are infected by a single administration of an LD_{100} dose of bacterial culture suspension ($1x10^8$ CFU/100µl per animal) in the tail vein. A careful clinical examination is made several times a day, and obvious clinical symptoms and mortality are recorded. Animals survival is observed for a period of 6 days. Azithromycin is dissolved in 0.5% methocel in saline solution and administered orally. Test compounds are micronized with mortar and pestle and then dissolved in methocel saline solution with 3% of DMF. The first dose is administered 30 minutes after infection, with following doses every 12 hours for 3 days.

***In vivo* efficacy testing – *S. aureus* mouse septicaemia model.**

Several compounds were tested in a S. aureus mouse protection models for efficacy. Two compounds, 25 and 62, protected 40% of treated animals from the lethal infection when administered orally.

Clinical Uses of the Heterocyclic Compounds

The compounds claimed in this invention can be used therapeutically or prophylactically for treatment or prevention of bacterial infections and/or diseases.

The invention also relates to methods of disrupting the internal regulation of microbial growth or respiration, in a subject, comprising the step of administering to said subject a compound of any of the formulae described herein or a composition comprising a compound of any of the formulae described herein. In one embodiment, the invention relates to a method of inhibiting microbial or bacterial activity in a subject comprising the step of administering a compound to the subject, or a composition comprising a compound, of any one of the formulae described herein. Preferably, the subject is a human being or animal.

In an alternate embodiment, this invention relates to a method of treating disease or disease symptoms in a subject comprising the step of administering to said subject a compound, or a composition comprising a compound, of any of the formulae described herein. Preferably, the subject is a human being or animal.

Infections and infectious diseases are caused from a variety of microorganisms. The compounds of the invention may find use in the medical treatment of infectious diseases from bacterial sources.

Compounds that kill or limit the growth of microorganisms may find use in the treatment of infections and infectious diseases. Specific bacterial microorganisms are known to be associated with the type of infection or infectious disease. Some examples of bacterial infections and their most common causative pathogens are given below.

Upper and lower respiratory tract infections include, but are not limited to: bronchitis, sinusitis, pneumonia, sore throat, chronic streptococcal infections, diphtheria, acute epiglottitis, influenza, chronic bronchitis, middle ear infections (otitis media), pneumonia, bronchopneumonia, Legionnaire's disease, atypical pneumonia, whooping cough, and tuberculosis.

Bacterial microorganisms causing respiratory tract infections include but are not limited to: *S.pyogenes, S.pneumoniae, S.aureus, H.influenzae, M.catarrhalis, N.meningitidis, B. pertussis,*, Enterobacteriaceae, Anerobes, Nocardia, Pseudomonas, C. psittaci, and C. diphtheriae.

Urinary tract infections include, but are not limited to: urethritis, cystitis, pyelonephritis (kidney infection), asymptomatic bacteruria, interstitial cystitis, acute urethral syndrome, and recurrent urinary tract infections.

Bacterial microorganisms causing urinary tract infections include but are not limited to: *E. coli, Proteus, Providentia, Pseudomonas, Klebsiella, Enterobacter, Serratia, Coag. neg. Staphylococci, Enterococci*, and *C. trachomatis*.

Skin and wound infections include, but are not limited to: erythrasma, panaritium, impetigo, folliculitis, erysipelas, cellulitis, and necrotizing fasciitis.

Bacterial microorganisms causing skin and wound infections include but are not limited to: *Streptococci, Staphylococci, P. aeruginosa, P. acnes, Clostridia, anaerobes, and B. fragilis*.

Bacterial microorganisms causing systemic infections (bacteremia) include but are not limited to: *Streptococci, Staphylococci, Enterobacteriaceae, Pseudomonas, Bacteroides sp., Neisseria, H. influenzae, Brucella, Listeria*, and *S. typhi*.

Sexually transmitted diseases of bacterial origin include, but are not limited to: adnexitis, cervicitis, chanchroid, urethritis, balanitis, gonorrhea, lymphogranuloma venereum, syphilis, and granuloma inguinale.

Bacterial microorganisms causing sexually transmitted infections include but are not limited to: *Chlamydia, N. gonorrhoeae, U. urealyticum, T. pallidium, G. vaginalis, H. ducreyi, C. granulomatis, Streptococci, Staphylococci*, and *Enterobacteriae*.

Gastrointestinal infections of bacterial origin include but are not limited to: food borne infections, colitis, enteritis, gastric ulcers, duodenal ulcers, pancreatitis, gall bladder infections, cholera, and thyphus.

Bacterial microorganisms causing gastrointestinal infections include but are not limited to: *H. pylori, C. pylori, C. duodeni, S. typhi, S. paratyphi, V. cholerae, anaerobes, Enterobacteriaceae, Staphylococci*, and *Streptococci*.

Methods of Treating Patients

The heterocyclic compounds of the formulae delineated herein can be administered to a patient, for example, in order to treat an infection such as a bacterial infection. The heterocyclic compounds can, for example, be administered in a pharmaceutically acceptable carrier such as physiological saline, in combination with other drugs, and/or together with appropriate excipients. The heterocyclic compounds of the formulae described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally,

buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a dosage ranging from about 0.001 to about 100 mg/kg of body weight, preferably dosages between 10 mg and 5000 mg/dose, every 4 to 120 hours, or according to the requirements of the particular drug. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In another embodiment, this invention provides methods of treating, preventing, or relieving symptoms of disease in a subject comprising the step of administrating to said subject any of the pharmaceutical compositions and combinations described above. Preferably, the subject is a human. If the pharmaceutical composition only comprises the compound of this invention as the active component, such methods may additionally comprise the step of administering to said subject an additional therapeutic agent such as, for example, a sulfa drug or a sulfonamide (e.g., sulfamethoxzole), macrolide antibiotics (e.g.,

clarithromycin), proton pump inhibitors (e.g., omeprazole), rifamycins (e.g., rifampin), aminoglycosides (e.g., streptomycin, gentamycin, , tobramycin), penicillins (e.g., penicillin G, penicillin V, ticarcillin), β-lactamase inhibitors, cephalosporins (e.g., cefazolin, cefaclor, ceftazidime), and antimycobacterial agents (e.g., isoniazid, ethambutol). Other suitable agents are delineated in infectious disease texts and publications, including for example, *Principles and Practice of Infectious Diseases*, G.L. Mandell et al. eds., 3rd ed., Churchhill Livingstone, New York, (1990). Such additional(s) agent may be administered to the subject prior to, concurrently with, or following the administration of the composition having a compound of any of the formulae described herein.

Pharmaceutical compositions of this invention comprise a compound of the formulae described herein or a pharmaceutically acceptable salt thereof; an additional agent selected from an anticancer agent, an anti-viral agent, antifungal agent, antibiotic, proton pump inhibitors, and any pharmaceutically acceptable carrier, adjuvant or vehicle. Alternate compositions of this invention comprise a compound of the formulae described herein or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier, adjuvant or vehicle. Such compositions may optionally comprise additional therapeutic agents, including, for example an additional agent selected from an anticancer agent, an antiviral agent, antifungal agent, antibiotic, and any pharmaceutically acceptable carrier, adjuvant or vehicle. Alternate compositions of this invention comprise a compound of the formulae described herein or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier, adjuvant or vehicle. Such compositions may optionally comprise additional therapeutic agents, including, for example an additional agent selected from an anticancer agent, an antimicrobial agent, an antiviral agent, antifungal agent, proton pump inhibitor, or antibiotic. The compositions delineated herein include the compounds of the formulae delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of microbial or bacterial levels.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers,

alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-β-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland

fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

Topical administration of the pharmaceutical compositions of this invention is especially useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and

water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The compounds and compositions of this invention are useful as sterilizants, antiseptics, adjuvants in wound dressings (e.g., bandages), and adjuvants in wound cleansing methods (swipes, gavage, etc.).

When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

Examples of potential application of combination therapies include compounds of any of the formulae delineated herein and the following: in combination with a macrolide antibiotic and a proton pump inhibitor for the treatment of gastritis and associated diseases caused by *Helicobacter pylori*; in combination with antibacterials (e.g., sulfamethoxazole, ciprofloxacin and amoxicillin) for the treatment of urinary tract infections;e.g. in combination with rifamycins for treatment of staphylococcal infections; e.g. in combination with rifamycins, isoniazid, ethambutol, or aminoglycosides for the treatment of mycobacterial infections; e.g. in combination with ticarcillin, gentamycin or tobramycin for the initial treatment of neutropenic patients with presumed infections, e.g. in combination

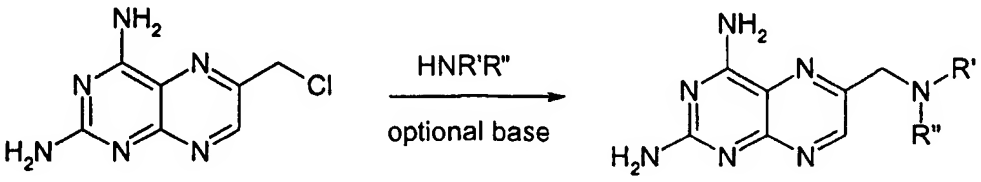
with penicillins or aminoglycosides for the treatment of enterococcal endocarditis; e.g. in combination with antibiotics for the treatment of intraperitoneal, pelvic or other polymicrobial infections; e.g. in combination with other DHFR inhibitors (e.g., trimethoprim, DHFR inhibitors delineated herein).

The invention will be further described in the following example. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

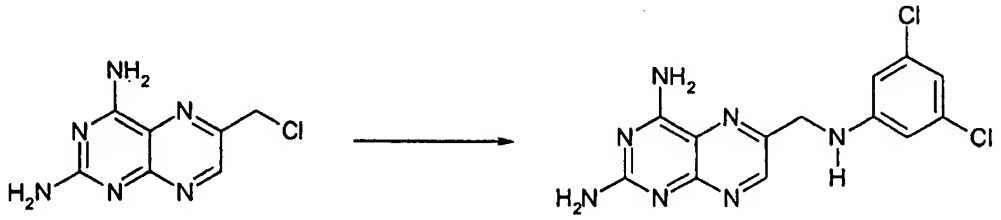
Examples

Liquid chromatographic data was obtained using a Hewlett-Packard (HP) 1090 Series Liquid Chromatograph coupled to a Diode Array Detector [Restek Allure C18 Column; particle size, 5μM; column length, 150mm; column diameter, 4.6 mm; flow rate, 1 mL/min; Solvent program, from 95% H_2O (w/ 0.1% TFA)/5% CH_3CN (w/ 0.1% TFA) to 100% CH_3CN (w/ 0.1% TFA) in 8 minutes, then held constant for 3 minutes; detection wavelength, 254 nm]. Mass Spectral data were obtained on either an Agilent 1100 LC/MS or Thermofinigan AQA/Gilson LC/MS system. ^1H- and ^{13}C-NMR spectra were obtained on a Bruker AC-300 MHz instrument. Medium pressure flash chromatography was performed on an Isco Inc., Combiflash Sg100c system. Thin-layer chromatography was performed using EM Science silica gel 60 F_{254} plastic TLC plates. Melting points were determined in open-air capillary tubes in a Meltemp II apparatus. UV light was used for detecting compounds on the TLC plates. Reagents used in reactions were purchased from the Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (Saint Louis, MO), Fluka Chemical Corp. (Milwaukee, WI), Fisher Scientific (Pittsburgh, PA), TCI America (Portland, OR), Transworld Chemicals, Inc. (Rockville, MD), Maybridge Chemical Ltd., (London, England) or Lancaster Synthesis (Windham, NH).

Synthesis of Pterin Analogs



In general 6-substituted pterin analogs can be prepared by the reaction of an activitated reagent such as 6-chloromethyl pterin with nucleophiles such as amines (e.g., HNR'R" can be HNHY or $HNR^{16}Y$). Other functional groups at the 6-position of pterin such as bromomethyl, iodomethyl, hydroxymethyl, activated hydroxymethyl, carbonyl (e.g., ketone or aldehyde), activated carbonyl (e.g., ester, amide, or anhydride), hydroxy, chloro, bromo, or methyl can be used as synthetic reagents for the preparation of 6-substitued analogs.

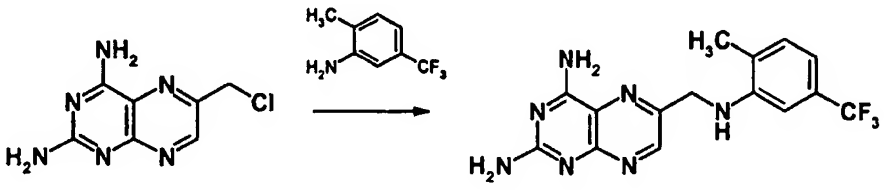


Synthesis of 6-(3,5-dichlorophenyl)aminomethyl-2,4-diaminopteridine

To a solution of 3,5-dichloroaniline (324 mg, 2 mmol) in dry DMF (5 mL) was added 2,4-diaminopteridine hydrochloride (100 mg, 0.4 mmol). The reaction vessel was flushed with dry nitrogen, sealed, and placed in an aluminum heating block at 85°C for 3 days. The solvent was then rotary evaporated. Ethanol was added (2 x 15 mL) and rotary evaporated repeatedly. To the resulting crude product was added H_2O (3 mL), HOAc (0.3 mL), and 1M HCl (0.1 mL) to pH ~2. The mixture was centrifuged to remove the insoluble impurities. The supernatant was removed and the pH adjusted to ~5 with 1M NaOH (~0.1 mL) to preciptate the product. This material was collected, washed with EtOH (3 mL), and dried under high vacuum to provide the purified product. HPLC rt 6.1 min, MS m/z.

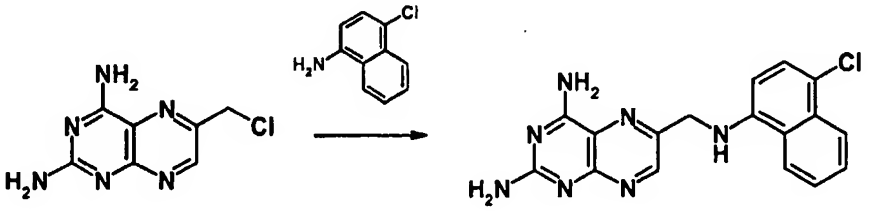
Nuclear magnetic resonance (NMR) spectroscopic analysis gave results consistent with the product.

Preparation of compound 64:



To a solution of 3-amino-4-methylbenzotrifluoride (3.35 g, 4.85 mmol) in DMF (10 mL) were added 2,4-diamino-6-chloromethylpteridine.HCl (1.57 g, 6.35 mmol) and K2CO3 (1.76 g, 12.7 mmol). The reaction mixture was purged with argon gas and heated to 60-65 C for 24 hours in a sealed vial with vigorous strirring. The reaction mixture was then allowed to cool to room temperature and poured into 0.5N HCl (~120 mL). The resulting mixture (pH 0-1; pH paper) was allowed to stir 15 minutes and then filtered. The collected precipitate was washed with 5% HCl, MeOH/CH2CL2, and Et2O. The product was dried under vacuum to provide the desired product (710 mg) as a yellow solid. This material was pure by RP-HPLC, Rt = 3.25 min, m/z = 350 (pos).

Preparation of compound 63:

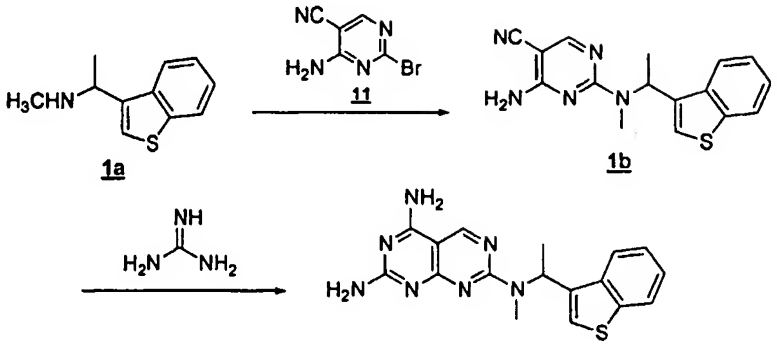


To a solution of 4-chloro-1-naphthyl amine (3.70 g, 20.8 mmol) in DMF (10 mL) were added 2,4-diamino-6-chloromethylpteridine.HCl (1.58 g, 6.39 mmol) and K2CO3 (1.81 g, 13.0 mmol). The reaction mixture was purged with argon gas and heated to 60-65 C for 34 hours in a sealed vial. The reaction mixture was then allowed to cool to room temperature and poured into excess 0.5N HCl. The pH of the resulting mixture was adjusted (NH4OH; pH 5; pH paper) and was then filtered. The collected precipitate was washed with 5H2O, MeOH/CH2CL2, and Et2O. The solid was suspended in Et2O and HCl (2N in Et2O, 6 mL) was added. The mixture was sealed and stirred for 0.5 hours. Evaporation, followed by drying under vacuum provided the product as a brown solid, which was ~90% pure by HPLC and showed a significant amount of impurities.

The brown solid was further purified by suspension in 0.5N HCl (120 mL) and conc. HCl (~5 mL) was added in an attempt to dissolve the material. Despite the high acidity (pH<0, pH paper) a significant amount of material remained undissolved. The precipitate was collected and washed with H2O, MeOH/CH2Cl2, and Et2O. The product was dried to provide 1.33 grams of the desired product as a red solid which had high purity by HPLC, Rt = 3.39 min; m/z = 353 (pos).

Synthesis of Pyrimidopyrimidine Analogs

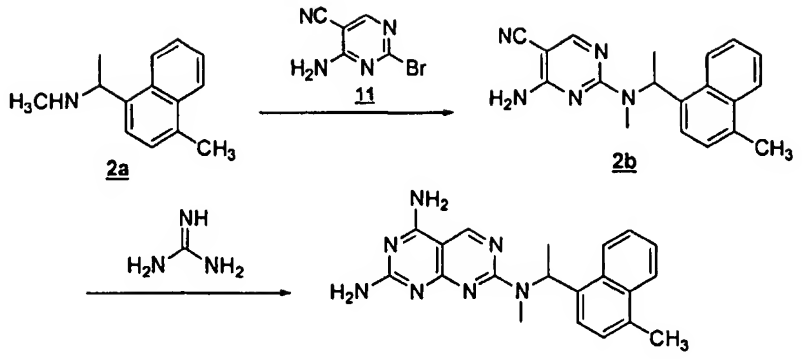
Preparation of Compound 25



A mixture of 2.89 g (14.5 mmol) pyrimidine **11**, 2.87 g (15 mmol) methylamine **1a** and 2.5 ml (18 mmol) triethylamine in 25 mL of 2-methoxyethanol was stirred at 80 °C for 2h. The reaction mixture was cooled down to room temperature and the solvent was evaporated to give a oily residue. 30 mL of ethyl acetate was added to dissolve the residue and the resulting solution was washed three times with water then dried over sodium sulfate. Evaporation gave an oily residue, which was then purified *via* the recrystallization from ether/ hexane. 3.95 g of product **1b** was obtained as white powder.

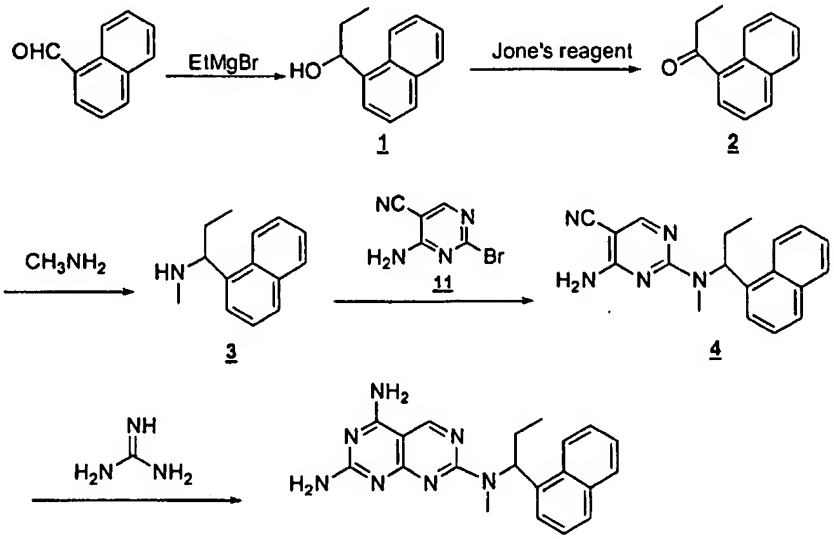
To a solution of 3.71 g **1a** in 40 mL of 2-methoxyethanol was added 24 mL of 1M guanidine in methanol and 16 mL of 1.5 M CH_3ONa in CH_3OH. The mixture was stirred at 140 °C for 12 h with an equipped Dean-Stark trap to remove the methanol solution. The reaction mixture was cooled down and evaporated *in vacuo* to give an oily residue, which was then dissolved in 30 mL of methanol. 50 mL of water was added to precipitate the product. The product was then purified by the recrystallization from methanol, then stirring in methanol three times. 920 mg of product was obtained as white powder. The purity of it was 98.52 % based on HPLC analysis.

Preparation of Compound 62



A similar procedures as for the preparation of compound **25** was used for the preparation of compound **62**. 860 mg of final product was obtained as white powder, which had 98.80 % of HPLC purity.

Procedures for the Preparation of Compound 33



Preparation of compound **1**:

To a solution of 4.25 g (27.2 mmol) naphthaldehyde in 30 ml dry ether in ice water bath was slowly added 13 ml of ethylmagnesium bromide, 3 M in ether. The mixture was stirred for another 30 min at room temperature and then quenched by adding 40 ml of 1 N HCl solution. The organic layer was washed with water (20 ml), sat. sodium bicarbonate (20 ml x

2), brine (20 ml) and then dried over anhydrous sodium sulfate. Evaporation of the organic solvent gave a crude product 1 which was directly used for the next step of the reaction without further purification

Preparation of compound 2:

The crude product 1 was dissolved in 30 ml acetone and to the resulting mixture, bathed in an ice water bath, was slowly added Jone's reagent until the brown color persisted. The solution was further stirred for 15 min at room temperature and then 5 ml of isopropanol was added. After 50 ml of ethyl acetate was added, the resulting mixture was washed with water (30 ml), sat. sodium bicarbonate (30 ml x 2), sat. NaCl and then dried over anhydrous sodium sulfate. Evaporation of the organic solvents gave an oily residue which was then purified by silica gel column chromatography. 4.01 g of ketone 2 was obtained.

Preparation of compound 3:

To the mixture of 4.01 g ketone 2 and 16.3 ml of methylamine in methanol, 2 M, was added 1.61 g sodium cyanoborohydride and 160 mg of zinc chloride. The resulting mixture was stirred overnight at 50 °C. Adding 1N HCl quenched the reaction. After most of the methanol was removed *in vacuo*, the solution was extracted with dichloromethane (15 ml x 2). The pH of the aqueous layer was adjusted to about 9 with 2 N NaOH. The product was then extracted with dichloromethane (15 ml x 3). The combined organic layer was washed with sat. NaCl and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave 3.54 g of compound 3.

Preparation of compound 4:

A mixture of 2.89 g (14.5 mmol) pyrimidine 11, 3.54 g (15 mmol) of compound 3 and 2.5 ml (18 mmol) triethylamine in 25 mL of 2-methoxyethanol was stirred at 80 °C for 2h. The resulting mixture was cooled down to room temperature and the solvent was evaporated to give an oily residue. 30 mL of ethyl acetate was added to dissolve the residue and the resulting solution was washed three times with water then dried over anhydrous sodium sulfate. Evaporation gave an oily residue, which was then purified by silica gel column chromatography. 3.95 g of product 4 was obtained as white powder.

Preparation of compound 33:

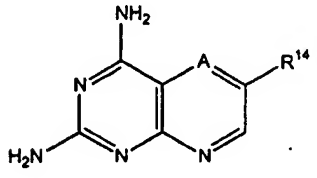
To a solution of 3.60 g of compound 4 in 40 mL of 2-methoxyethanol was added 24 mL of 1M guanidine in methanol and 16 mL of 1.5 M CH_3ONa in CH_3OH. The mixture was stirred at 140 °C for 12 h with an equipped Dean-Stark trap to remove the methanol solution. The reaction mixture was cooled down and evaporated *in vacuo* to give an oily residue, which was then dissolved in 30 mL of methanol. 50 mL of water was added to precipitate the product. The product was then purified by recrystallization from methanol, and the recrystallized product was then stirred in methanol three times. 1.95 g of the product was obtained as white powder. The purity of it was greater than 99% based on HPLC analysis.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

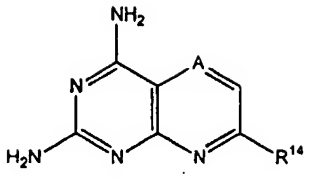
WHAT IS CLAIMED IS:

1. A compound of the following formulae:



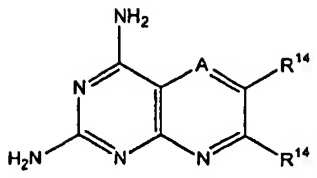
I

or



II

or



III

wherein

A is N, CH, or CR^{15};

R^{14} is $-(CH_2)_n-X-Y$, wherein

n is 1, 2, 3, 4, 5, or 6;

X is O, NH, or NR^{15}; and

Y is aryl or heteroaryl, wherein each Y is optionally substituted with 1-4 independent R^{16};

each R^{15} is independently lower alkyl;

each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $S(O)_2NR^{17}R^{17}$, $S(O)_2R^{17}$, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$;

each R^{17} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$;

each R^{18} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy,

trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$;

each R^{19} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$;

each R^{20} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$; and

each R^{21} is independently hydrogen, alkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, or heteroarylalkoxy.

2. The compound of claim 1, wherein A is N.

3. The compound of claim 1, wherein n is 1.

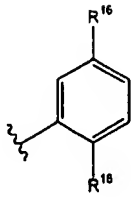
4. The compound of claim 1, wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}.

5. The compound of claim 3, wherein X is O.

6. The compound of claim 5, wherein A is N.

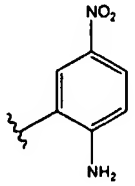
7. The compound of claim 6, wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}.

8. The compound of claim 7, wherein Y is



9. The compound of claim 8, wherein each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$.

10. The compound of claim 9, wherein Y is



11. The compound of claim 5, wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}.

12. The compound of claim 11, wherein phenyl is substituted with 1-4 independent R^{16}.

13. The compound of claim 3, wherein X is NH or NR^{15}, and R^{15} is CH_3.

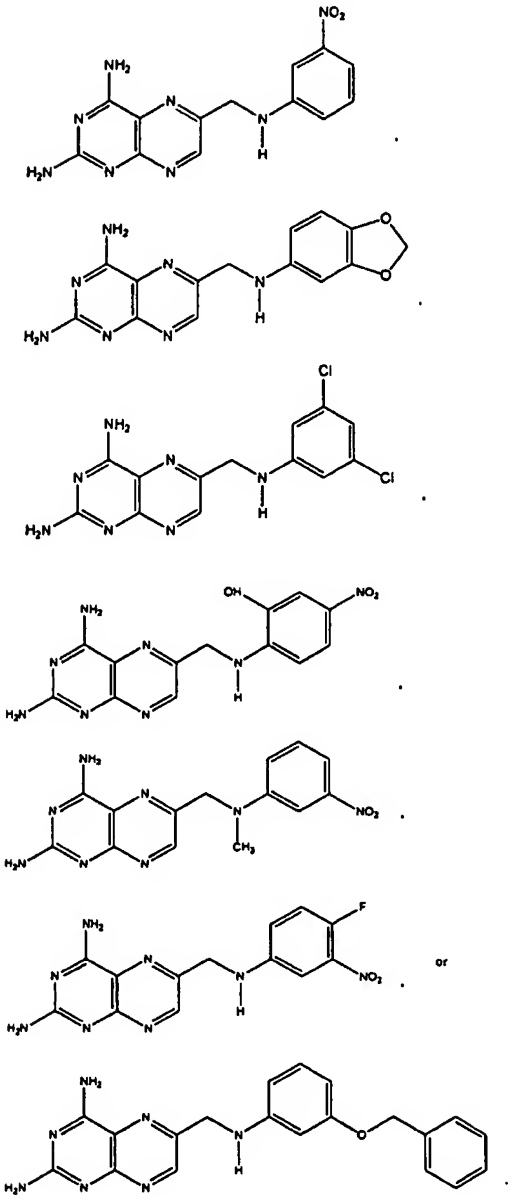
14. The compound of claim 13, wherein A is N.

15. The compound of claim 14, wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}.

16. The compound of claim 15, wherein Y is phenyl.

17. The compound of claim 15, wherein each R^{16} is independently halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$.

18. The compound of claim 17, wherein the compound is



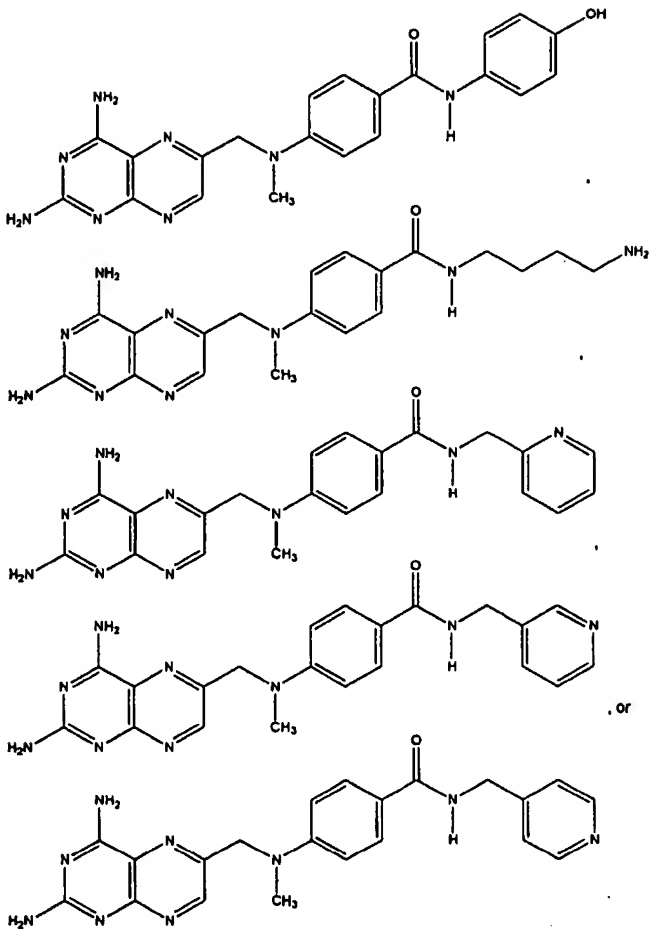
19. The compound of claim 17, wherein each R^{16} is $C(O)OR^{18}$.

20. The compound of claim 19, wherein each R^{18} is H or alkyl.

21. The compound of claim 20, wherein each R^{18} is CH_3.

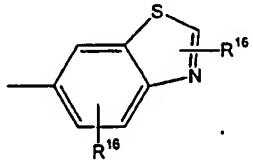
22. The compound of claim 17, wherein each R^{16} is $C(O)NR^{19}R^{20}$.

23. The compound of claim 22, wherein the compound is



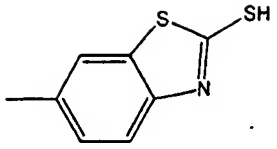
, or

24. The compound of claim 14, wherein Y is



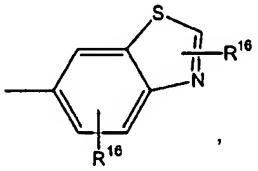
optionally substituted with 1-4 independent R^{16}, each R^{16} being halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$.

25. The compound of claim 24, wherein Y is



26. The compound of claim 13, wherein Y is phenyl, optionally substituted with 1-4 independent R^{16}.

27. The compound of claim 13, wherein Y is



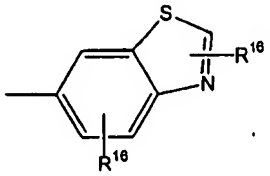
optionally substituted with 1-4 independent R^{16}, each R^{16} being halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)OR^{18}$, $C(O)NH_2$, or $C(O)NR^{19}R^{20}$.

28. The compound of claim 4, wherein A is N.

29. The compound of claim 4, wherein Y is phenyl, substituted with 1-4 independent R^{16}.

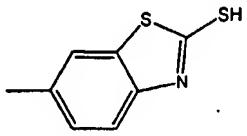
30. The compound of claim 4, wherein Y is phenyl.

31. The compound of claim 1, wherein Y is



optionally substituted with 1-4 independent R^{16}, wherein each R^{16} is halo, alkyl, alkylenedioxo, aryl, aralkyl, nitro, hydroxy, mercapto, amine, alkoxy, arylalkoxy, or heteroarylalkoxy.

32. The compound of claim 31, wherein Y is



33. The compound of claim 1, wherein A is CH or CR^{15}.

34. The compound of claim 1, wherein each R^{16} is independently $C(O)NR^{19}R^{20}$.

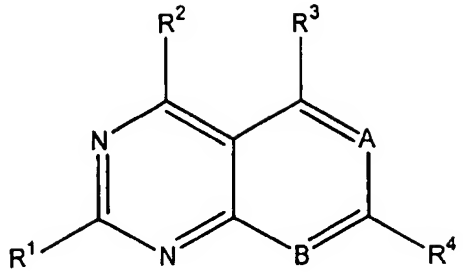
35. The compound of claim 34, wherein each R^{19} is independently aminoalkyl, aryl, heteroaryl, arylalkyl, or heteroarylalkyl; and each R^{20} is independently aminoalkyl, aryl, or arylalkyl; each aryl or heteroaryl is optionally substituted with 1-4 independent halo, cyano, alkyl, trifluoromethyl, hydroxyalkyl, alkylenedioxo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, nitro, hydroxy, mercapto, amino, alkoxy, thioalkoxy, trifluoromethoxy, aryloxy, heteroaryloxy, arylalkoxy, heteroarylalkoxy, $C(O)R^{15}$, $C(O)OR^{21}$, $C(O)NR^{21}R^{21}$, $S(O)_2NR^{21}R^{21}$, or $S(O)_2R^{21}$.

36. A composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

37. A composition comprising a compound of claim 1, an additional therapeutic agent, and a pharmaceutically acceptable carrier.

38. A composition comprising a compound of claim 1, an additional therapeutic agent, and a pharmaceutically acceptable carrier, wherein the additional therapeutic agent is an antibacterial agent.

47. A compound comprising the formula:



wherein

A and B are independently selected from the group consisting of N and CR^7, wherein R^7 is hydrogen or a carbon-, nitrogen-, sulfur- halogen- and/or oxygen-containing function group;

R^1 and R^2 are $-NR^5R^6$ groups, wherein R^5 and R^6 are independently hydrogen or carbon-containing functional groups;

R^3 is hydrogen;

R^4 is a carbon-, nitrogen-, sulfur-, halogen-, and/or oxygen-containing functional group,

provided that,

if A and B are both nitrogen and R^5 and R^6 are both hydrogen, then R^4 is not $-NH_2$, -N(H)(methyl), -N(H)(butyl), -N(H)(hexyl), -N(H)(phenyl), -N(H)(benzyl), $-N(H)(NH_2)$, $-N(H)(CH_2CH_2OH)$, $-N(CH_2CH_2OH)_2$, phenyl, N-piperidinyl, or -S(ethyl);

if A is CH, B is nitrogen, and R^5 and R^6 are both hydrogen, then R^4 is not methyl, isobutyl, phenyl, 4-methylphenyl, 4-chlorophenyl, 4-bromophenyl, 2-(2,5-dimethoxyphenyl)-ethyl, or $-CH(OCH_3)_2$; and

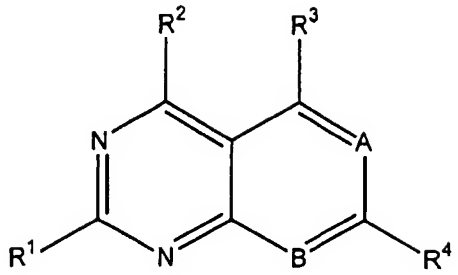
if A and B are both CH groups, then R^4 is an amino group other than $-NH_2$, (3,4-dichlorophenyl)methylamino, or (3,4-dichlorophenyl)methyleneimino.

48. The compound of claim 47 wherein,

R^5 and R^6 are independently hydrogen; and

A and B are each independently N.

47. A compound comprising the formula:



wherein

A and B are independently selected from the group consisting of N and CR^7, wherein R^7 is hydrogen or a carbon-, nitrogen-, sulfur- halogen- and/or oxygen-containing function group;

R^1 and R^2 are $-NR^5R^6$ groups, wherein R^5 and R^6 are independently hydrogen or carbon-containing functional groups;

R^3 is hydrogen;

R^4 is a carbon-, nitrogen-, sulfur-, halogen-, and/or oxygen-containing functional group,

provided that,

if A and B are both nitrogen and R^5 and R^6 are both hydrogen, then R^4 is not $-NH_2$, -N(H)(methyl), -N(H)(butyl), -N(H)(hexyl), -N(H)(phenyl), -N(H)(benzyl), $-N(H)(NH_2)$, $-N(H)(CH_2CH_2OH)$, $-N(CH_2CH_2OH)_2$, phenyl, N-piperidinyl, or -S(ethyl);

if A is CH, B is nitrogen, and R^5 and R^6 are both hydrogen, then R^4 is not methyl, isobutyl, phenyl, 4-methylphenyl, 4-chlorophenyl, 4-bromophenyl, 2-(2,5-dimethoxyphenyl)-ethyl, or $-CH(OCH_3)_2$; and

if A and B are both CH groups, then R^4 is an amino group other than $-NH_2$, (3,4-dichlorophenyl)methylamino, or (3,4-dichlorophenyl)methyleneimino.

48. The compound of claim 47 wherein,

R^5 and R^6 are independently hydrogen; and

A and B are each independently N.

39. A method of for prophylaxis of bacterial infection and or treatment of a bacterially infected organism, comprising administering to the organism an effective amount of a compound of claim 1.

40. A method for treating infection in a mammal comprising administration of the composition of claim 36.

41. A method of making a compound of claim 1, comprising taking a 2,4-diamino pteridinyl compound and reacting it with one or more chemical reagents in one or more steps to produce a compound of claim 1.

42. A product made by the method of claim 41.

43. A method of making a compound of claim 1, comprising taking any one of the intermediate compounds described herein and reacting it with one or chemical reagents in one or more steps to produce a compound of claim 1.

44. A product made by the method of claim 43.

45. A method for identifying a compound having antibacterial activity, comprising:

a) assessing the structure of a compound of claim 1;

b) procuring a derivative compound of the compound from step a); and

c) assessing the antibacterial activity of the derivative compound.

46. A method for identifying a compound having antibacterial activity comprising:

a) taking a candidate compound of claim 1;

b) assessing the binding affinity of the candidate compound in a model of the hDHFR enzyme; and

c) assessing the antibacterial activity of the candidate compound.

49. The compound of claim 48 wherein,

R^4 is NR^7R^8;

R^7 is C1-C6 alkyl substituted with aryl or heteroaryl;

R^8 is C1-C6 alkyl optionally substituted with alkenyl, hydroxyl, alkoxy, cycloalkyl, or aryl.

50. The compound of claim 49 wherein,

R^7 is C1-C6 alkyl substituted with aryl;

R^8 is C1-C6 alkyl.

51. The compound of claim 49 wherein,

R^7 is C1-C6 alkyl substituted with heteroaryl;

R^8 is C1-C6 alkyl.

52. The compound of claim 49 wherein,

R^7 is C1-C6 alkyl substituted with aryl;

R^8 is C1-C6 alkyl substituted with hydroxyl or alkoxy.

53. The compound of claim 48 wherein,

R^4 is NR^7R^8;

R^7 is C1-C6 alkyl;

R^8 is C1-C6 alkyl substituted with cycloalkyl.

54. The compound of claim 48 wherein,

R^4 is NR^7R^8;

R^7 is independently C1-C6 alkyl substituted with aryl;

R^8 is independently C1-C6 alkyl substituted with aryl.

55. The compound of claim 48, wherein

R^7 is hydrogen, alkyl, cycloalkyl, aryl, halogen, thioalkyl, hydroxy, alkoxy, amino, alkyl, or NR^5R^6; wherein each R^5 and R^6 on the nitrogen atom independently is hydrogen, alkyl, cycloalkyl, aryl, arylalkyl, heteroarylalkyl, or alkylcarbonyl.

56. The compound of claim 50, wherein R^7 is C1-C6 alkyl substituted with naphthyl, which is optionally substituted with alkyl, halo, hydroxy, alkoxy, thioalkyl, or amino.

57. The compound of claim 56, wherein the naphthyl group is substituted at the 2- or 4-position.

58. The compound of claim 57, wherein R^8 is methyl or ethyl.

59. The compound of claim 51, wherein R^7 is C1-C6 alkyl substituted with benzothienyl, which is optionally substituted with alkyl, halo, hydroxy, alkoxy, thioalkyl, or amino.

60. The compound of claim 59, wherein R^8 is methyl or ethyl.

61. A composition comprising a compound of claim 47 and a pharmaceutically acceptable carrier.

62. A composition comprising a compound of claim 47, an additional therapeutic agent, and a pharmaceutically acceptable carrier.

63. A composition comprising a compound of claim 47, an additional therapeutic agent, and a pharmaceutically acceptable carrier, wherein the additional therapeutic agent is an antibacterial agent.

64. A method of for prophylaxis of bacterial infection and or treatment of a bacterially infected organism, comprising administering to the organism an effective amount of a compound of claim 47.

65. A method for treating infection in a mammal comprising administration of the composition of claim 61.

66. A method of making a compound of claim 47, comprising taking a 2,4-diamino pyrimidopyrimidinyl compound and reacting it with one or more chemical reagents in one or more steps to produce a compound of claim 47.

67. A product made by the method of claim 66.

68. A method of making a compound of claim 47, comprising taking any one of the intermediate compounds described herein and reacting it with one or chemical reagents in one or more steps to produce a compound of claim 47.

69. A product made by the method of claim 68.

70. A method for identifying a compound having antibacterial activity, comprising:

a) assessing the structure of a compound of claim 47;

b) procuring a derivative compound of the compound from step a); and

c) assessing the antibacterial activity of the derivative compound.

71. A method for identifying a compound having antibacterial activity comprising:

a) taking a candidate compound of claim 47;

b) assessing the binding affinity of the candidate compound in a model of the hDHFR enzyme; and

c) assessing the antibacterial activity of the candidate compound.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 January 2003 (09.01.2003)

PCT

(10) International Publication Number
WO 03/002064 A3

(51) International Patent Classification[7]: C07D 487/04, 471/04, 239/95, A61K 31/519, 31/517, A61P 31/04

(21) International Application Number: PCT/US02/20479

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/301,685 28 June 2001 (28.06.2001) US

(71) Applicant *(for all designated States except US)*: **ESSENTIAL THERAPEUTICS, INC.** [US/US]; 1365 Main Street, Waltham, MA 02451-1624 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MOE, Scott, T.** [US/US]; Three Carver Hill Road, Marlborough, MA 01752 (US). **CLEMENT, Jacob, J.** [US/US]; 74 Golden Run Rd., Bolton, MA 01740 (US). **FAERMAN, Carlos** [US/US]; 41 Mohawk Drive, Acton, MA 01720 (US). **PEROLA, Emanuela** [IT/US]; 127 Second Street, Apt. 2, Cambridge, MA 02141 (US). **NAVIA, Manuel, A.** [US/US]; 21 Washington Street, Lexington, MA 02421 (US). **ALA, Paul, J.** [CA/US]; 122 Bowdoin Street, Apt. 23, Boston, MA 02108 (US). **MAGEE, Andrew, S.** [US/US]; 21 Maple Street, Maynard, MA 01754 (US). **WILL, Paul, M.** [US/US]; 29 Pearl Street, Lunenburg, MA 01462 (US). **MARCHESE, Salvatore, A.** [—/US]; 32 Vining Street, Malden, MA 02148 (US). **GAZZANIGA, John, V.** [US/US]; Six Dominion Road, Worcester, MA 01605 (US).

(74) Agent: **HSI, Jeffrey, D.**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
23 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/002064 A3

(54) Title: FUSED PYRIMIDINE DHFR INHIBITORS AS ANTIBACTERIALS

(57) Abstract: This invention relates to novel heterocyclic compounds and to their use in the medical treatment or prophylaxis of bacterial infections, or their use as antiseptics, sterilizants, or disinfectants.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/20479

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07D 487/04, 471/04, 239/95; A61K 31/519, 31/517; A61P 31/04

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/260, 514/249

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 4079056A (PIPER et al.) 14 March 1978. See col. 17, compounds 11-15, 18-19, 21, 29-30.	1-7, 11-17, 19-22, 26, 28-30, 34-37, 41-44 --- 8-9 --- 10, 18, 23, 24-25, 27, 31-32. 38-40. 45-46

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 MARCH 2003	Date of mailing of the international search report 02 MAY 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Valerie Bell-Harris for USPTO/Mark L. Berch/jmr Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/20479

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

544/260, 279, 284, 291, 256; 514/262.1, 264.11, 266.2, 266.4, 249

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, Pteridines, A=N in claim 1, drawn to claim 1 (part), 2, 3-5 (part), 6-10, 11-13 (part), 14-25, 26-27 (part), 28, 29-32 (part), 34-46 (part).
Group II, claim(s) Pyridopyrimidines, drawn to A=C in claim 1, or exactly one of A, B is N in claim 47, drawn to claim 1 (part), 11-13 (part), 26-27 (part), 29-32 (part), 33, 34-47 (part), 61-71 (part).
Group III , claim(s) Pyrimidopyrimidines, A=B in claim 47, drawn to claim 47 (part), 48-60, 61-71 (part).

Group IV, claim Quinazolines, A=B=C in claim 47, drawn to claim 47 (part), 61-71 (part).

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each group has a special technical feature not seen in the others Viz., it is heterocyclic core. Thus, in Group IV it is the quinazoline ring, the only one with only 2 nitrogen present. For Group VII it is the pyrimidopyrimidine, the only group with a ring system with 4 nitrogens. Group II is the pyridopyrimidines, the only one with an azine ring.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/20479

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	TAYLOR, EDWARD C. et al. "Synthesis of some 2 4-diamino-6-substituted methylpteridines" Journal of Organic Chemistry 40 (16), 2347 (1975). See 6e, h, j, k, l, t, u.	1-7, 11-17, 19-21, 26, 28-30, 41-44 ---- 8-10. 18. 22-25. 27, 31-32, 34-40, 45-46
X ---- Y	CHAYKOVSKY, MICHAEL et al. "Methotrexate Analogs 3. Synthesis and biological properties of some side-chain altered analogs" Journal of Medicinal Chemistry, 1974. 17(11) 1212-1216. See 6 c, e, f.	1-4, 6, 7, 11-17, 22, 26, 28-30. 33-34, 36, 39-46 ---- 37-38
X ---- Y	PIPER et al. "Lipophilic Antifolates as Agents against Opportunistic Infections. 1, Agents Superior to Trimetrexate and Piritrexim against Toxoplasma gondii and Pneumocystis carinii in Vitro Evaluations" Journal of Medicinal Chemistry. 1996, Vol. 39 (6), 1271-1280. See Table 2, 12a, 12k, 12l, 12m, 12n, 12o, 12p, 12q, 12r, 12s.	1-4, 6-8. 11-17, 26, 28-30, 36, 39-46 ---- 9, 18, 37-38
X ---- Y	WORTH, DANIEL et al. "Antimalarial drugs. 38. Folate antagonists 10. Synthesis and antimalarial effects of 6[[(aryl and aralkyl)amino]methyl-2,4-pteridinediamones and -pteridinediamine 8-oxides". Journal of Medicinal Chemistry. 1978. Vol. 21(4), 331-337. See 39-48.	1-4, 6-7, 11-17, 26, 28-30, 36, 39-46 ---- 8, 9, 18.